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Micronutrient Profiles in HIV-1-Infected Heterosexual Adults

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Summary: There is compelling evidence that micronutrients can profoundly affect immunity. We surveyed vitamin supplement use and circulating concentrations of 22 nutrients and glutathione in 64 HIV-1 seropositive men and women and 33 seronegative controls participating in a study of heterosexual HIV-1 transmission. We assayed antioxidants (vitamins A, C, and E; total carotenes), vitamins B_6 and B_{12}, folate, thiamin, niacin, biotin, riboflavin, pantothenic acid, free and total choline and carnitine, biopterin, inositol, copper, zinc, selenium, and magnesium. HIV-infected patients had lower mean circulating concentrations of magnesium ($p < 0.0001$), total carotenes ($p = 0.009$), total choline ($p = 0.002$), and glutathione ($p = 0.045$), and higher concentrations of niacin ($p < 0.0001$) than controls. Fifty-nine percent of HIV+ patients had low concentrations of magnesium, compared with 9% of controls ($p < 0.0001$). These abnormal concentrations were unrelated to stage of disease. Participants who took vitamin supplements had consistently fewer low concentrations of antioxidants, across HIV infection status and disease stage strata ($p = 0.0006$). Nevertheless, 29% of the HIV+ patients taking supplemental vitamins had subnormal levels of one or more antioxidants. The frequent occurrence of abnormal micronutrient nutriture, as found in these HIV+ subjects, may contribute to disease pathogenesis. The low magnesium concentrations may be particularly relevant to HIV-related symptoms of fatigue, lethargy, and impaired mentation. Key Words: Micronutrient—HIV-1 infection—Trace metal—Immunity.

Deficiencies of single micronutrients are known to adversely affect the immune system by depression of cellular and humoral immunity and impairment of phagocytosis (1,2). Individuals infected with the human immunodeficiency virus type 1 (HIV-1) may be particularly vulnerable to nutritional deficiencies that impair already compromised immune function. In a previous study of HIV-1 infected patients, we found that carotenes and ascorbate were below normal in 27% of the subjects, and vitamins E and A were low in 12% (3). Serum levels of micronutrients in HIV-1 patients have been associated with markers of immune function and stage of disease (4–7). Studies have shown that abnormalities in nutriture both accompany and predict HIV disease progression (6,8–10). These investigations assessed dietary intake or serum concentrations of one or a few micronutrients in selected cohorts. In

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this study, we surveyed concentrations of 22 circulating micronutrients and glutathione among 64 HIV-1 infected outpatients, ranging from asymptomatic to AIDS-diagnosed, and 33 seronegative controls. We also examined the association of self-directed vitamin supplementation on micronutrient status. Our data may provide insight for making clinical decisions and for understanding disease progression.

PATIENTS AND METHODS

Study Subjects

Between June 1992 and March 1993, HIV-1 infected and uninfected subjects were recruited from couples enrolled in a longitudinal study of heterosexual HIV-1 transmission conducted at the UMDNJ-New Jersey Medical School in Newark, New Jersey. Couples were referred by infectious diseases clinics, physicians, and New Jersey counseling and testing sites. Procedures are further described elsewhere (11). The same clinics and physicians referred additional HIV-1 infected patients, and additional seronegative controls were recruited from local staff. We obtained informed consent under a protocol approved by the New Jersey Medical School Institutional Review Board.

We determined HIV-1 serostatus by enzyme-linked immunosorbent assay and confirmed the status by Western blot. Participants provided information by structured interview on demographic background, clinical condition, weight, and on any medications, vitamins, and nutritional supplements they were currently taking. (The study protocol neither advised nor restricted the use of supplements.) Clinical stage of disease was assigned by the Centers for Disease Control 1993 Revised Classification System (12). For the seropositive subjects enrolled in the heterosexual transmission study, lymphocyte subpopulations were determined at 6-month intervals; for the remaining seropositive subjects, CD4+ and CD8+ counts were obtained from recent clinical records.

Blood Collection and Analysis

Blood for vitamin determinations was collected in lavender-top Vacutainers (Becton, Dickinson & Co., Rutherford, NJ, U.S.A.) with EDTA as an anticoagulant. Plasma for trace element analyses was collected in heparinized metal-free Vacutainers. Blood samples obtained at routine clinical follow-up were delivered to the laboratories ≤4 h of collection. Plasma concentrations of vitamins A, C, E, and carotenes were determined spectrophotometrically (13). Plasma concentrations of vitamin B_6 were analyzed with protozoa, and *Lactobacillus casei* was used for folate assays of plasma (13). Whole blood was analyzed with various protozoa for thiamin, biotin, nicotinates (niacin), pantothenates, vitamins B_6 and B_{12}, and riboflavin (13). Plasma carnitine, choline, biopterin, and inositol determinations were carried out by previously described methods (14–17). Plasma concentrations of zinc, copper, and magnesium were measured by flame atomic absorption spectrophotometry (18,19); plasma selenium was measured by electrothermal atomic absorption (20).

Determination of glutathione required that subjects schedule a second blood draw at our laboratory, because the assay required immediate processing. Plasma was collected in metal-free Vacutainers containing EDTA and centrifuged, transferred to a polypropylene tube, mixed with 10% 5-sulfosalicylic acid, and centrifuged (21). Glutathione concentrations in the supernatant were determined by visible spectrophotometry at 412 nm (modified Beckman DB-GT). Vitamin and trace element assays were also repeated on the blood collected at this visit, to better characterize the micronutrient status of individuals.

Statistics

Measured concentrations were approximately normally distributed. To accommodate the correlation of duplicate determinations on the same subject, we estimated group mean concentrations by least square means and compared them by repeated measures analysis of variance (ANOVA), using SAS (SAS Institute Inc., Cary, NC, U.S.A.) software. Concentrations of all micronutrients except total choline were defined as *abnormal* if they were outside the laboratory's established normal range of mean ± 2 SDs for a reference population of ≥300 healthy adults. The normal range for total choline was based on the range of the seronegative controls. Group prevalences of out-of-range concentrations were based on determinations at the first visit and compared using StatXact@ (Cytel Software Corp., Cambridge, MA, U.S.A.) exact contingency table analyses. The subject was the unit of analysis. Associations between micronutrient concentrations and lymphocyte counts were described by Pearson correlation coefficients. We performed stepwise multiple regression to evaluate the joint effects of micronutrients on lymphocyte counts; final models included correlates after backward elimination at $p \geq 0.10$. All p values are two-tailed; $p < 0.05$ is considered statistically significant.

RESULTS

We obtained micronutrient data from 97 study subjects. Sixty-nine subjects (52 seropositive, 17 seronegative controls) provided blood samples at two visits, for a total of 166 micronutrient determinations. The interval between visits ranged from 28 to 126 days (median 49 days).

Subject characteristics are presented in Table 1 by infection status and stage of disease. Sixty-four (65%) subjects were seropositive for HIV-1 infection: 19 were diagnosed with AIDS, 18 had HIV-related symptoms, and 27 remained asymptomatic. Forty-three HIV-positive subjects (67%) and 17 seronegative controls (52%) were male. No female was pregnant. Sixty percent of the subjects were white non-Hispanic (33 HIV-positive, 25 controls); 26% were Hispanic (21 HIV-positive, 5 controls); 13% were black non-Hispanic (10 HIV-positive, 2 controls); and 1 control was Asian. Seronegative controls were more predominantly white (76 vs. 52%); approximately two-thirds of nonwhite sub-

TABLE 1. *Characteristics of subjects at first visit*

Diagnostic category	N	Male (%)	Age (yr) Mean ± SEM	$CD4^+$ in cells/ml Mean ± SEM	Percent taking vitamin supplements
HIV seronegative	33	52	40 ± 1.8		42
HIV seropositive CDC stage*					
A (asymptomatic)	27	67	37 ± 1.5	508 ± 51	63
B (symptomatic)	18	61	36 ± 2.0	208 ± 35	73
C (AIDS)	19	74	37 ± 1.6	222 ± 54	63

CDC, Centers for Disease Control.

jects in both groups were Hispanic. Controls were slightly but not significantly older: mean 40 versus 36 years. Twenty-five male and three female seropositive subjects were i.v. drug users; three men were bisexual. Fifteen men and 18 women attributed their infection to heterosexual transmission.

HIV-infected subjects reported taking antiviral medication at the time of 82 (71%) of their 116 visits. Concurrent prophylaxis for *Pneumocystis carinii* pneumonia was infrequently reported (at 16 visits). Nine patients (14%) reported a weight loss of ≥5 kg in the past 6 months (maximum: 12 kg), and none showed clinical evidence of wasting (weight loss >10% in 6 months, with recurrent fever or diarrhea). All HIV-infected subjects were clinically stable outpatients.

Intake of Supplemental Vitamins

Current consumption of multivitamin supplements was reported at 98 of the 166 subject visits (59%). Supplemental consumption of vitamin C was reported at 40 visits (24%), of vitamin E at 32 (19%), and of B-complex at 23 (14%). These vitamins were taken in various combinations, typically in addition to a multivitamin supplement. Ten subjects reported taking vitamin supplements at only one of their two visits (5 at the first, 5 at the second). Four subjects took supplements other than vitamins (1: Ca, Mg; 1: protein, iron; 2: phosphorus). HIV-infected subjects reported taking some vitamin supplement at 66% of their 116 study visits, compared to 46% of 50 visits by controls.

HIV Infection Status and Micronutrient Levels

Mean Micronutrient Levels

Mean plasma concentrations of micronutrients are presented by serostatus in Table 2. Concentrations of antioxidants (vitamins A, C, E, total carotenes, and glutathione) tended to be lower among those with HIV infection than among seronegative controls; the difference was significant in repeated measures ANOVA only for carotenes ($p = 0.009$) and glutathione ($p = 0.045$). Levels of the vitamin B complex and related metabolites were comparable for HIV-infected and uninfected subjects, except that niacin was significantly higher among HIV-infected patients ($p < 0.0001$) and total choline was lower ($p < 0.002$). Concentrations of magnesium were also lower in HIV-infected subjects ($p < 0.0001$), but levels of zinc, selenium, and copper did not differ between infected and uninfected subjects.

In analyses of HIV-infected subjects classified by stage of illness, mean concentrations of micronutrients did not vary systematically with increasing severity of illness.

Abnormal Micronutrient Levels

Table 2 presents the proportion of micronutrient concentrations that fell below and above the normal laboratory range. These frequencies are based on 166 determinations that included replicates for subjects with two visits. The frequencies based solely on the first visit are similar and were used for hypothesis testing of group differences.

At the first visit, significantly more HIV-1 infected subjects had low concentrations of magnesium (59 vs. 9%, $p < 0.0001$) and carotenes (25 vs. 3%, $p = 0.009$) than uninfected controls. Low concentrations of vitamin C (20%) and folic acid (15%) were not more prevalent among HIV-infected patients than among controls.

We investigated the possibility that low magnesium concentrations among the HIV-infected cohort reflected a greater number of heavy drinkers (≥3 drinks a day). HIV infection remained a significant predictor of low magnesium but heavy drinking was not, when the factors were considered

TABLE 2. *Circulating micronutrient concentrations in HIV-infected and seronegative subjects*[a]

	Normal range	Unit	Percentage beyond normal range: Below range (%) HIV +	Below range (%) HIV −	Above range (%) HIV +	Above range (%) HIV −	Least squares mean ± SE: HIV +	Least squares mean ± SE: HIV −	p Value[b]
Vitamin A	0.87–2.62	μmol/L	0	0	23	35[e]	2.13 ± 0.10	2.41 ± 0.15	0.10
Vitamin C	23–85	μmol/L	20	10	7	8	44.9 ± 3.1	48.8 ± 4.8	
Vitamin E	14–35	μmol/L	4	0	21	26	29.3 ± 1.7	33.9 ± 2.6	0.11
Total carotenes	1.5–5.6	μmol/L	26	2[d]	3	4	2.40 ± 0.20	3.33 ± 0.32	0.009
Glutathione[f]							4.71 ± 0.27	5.58 ± 0.33	0.045
Vitamin B_{12}	200–1390	pmol/L	2	0	3	2	573 ± 40	450 ± 64	0.08
Folic acid	11–54	nmol/L	15	16	25	18	38.5 ± 4.3	34.0 ± 6.8	
Vitamin B_6	179–479	nmol/L	2	0	18	8	389 ± 42	317 ± 67	
Thiamin	0.07–0.21	μmol/d	0	0	5	4	0.163 ± .004	0.148 ± .007	0.08
Niacin	28–57	μmol/L	4	8	9	0[e]	43.9 ± 0.89	37.4 ± 1.38	0.0001
Biotin	820–3070	pmol/L	0	0	25	29	2840 ± 143	2820 ± 94	
Riboflavin	265–1330	nmol/L	0	0	9	8	954 ± 27	988 ± 43	
Pantothenic acid	0.91–4.56	μmol/L	0	0	4	4	2.28 ± 0.12	1.96 ± 0.20	0.15
Free choline	3.2–6.2	μg/ml	0	0	17	7[e]	4.9 ± 0.11	4.8 ± 0.18	
Total choline	170–665	μg/ml	4	2	1	2	300 ± 11.6	364 ± 18.4	0.002
Biopterin	3.0–7.2	nmol/L	0	2	28	26	6.3 ± 0.21	5.9 ± 0.38	
Inositol	17–67	μmol/L	4	2	1	0	29.4 ± 1.0	32.2 ± 1.6	0.11
Free carnitine	29–49	μmol/L	0	4	37	48	47.8 ± 1.4	49.6 ± 2.2	
Total carnitine	33–61	μmol/L	0	2	35	38	58.3 ± 1.6	55.8 ± 2.5	
Copper	10.2–22.8	μmol/L	0	2	0	8[e]	16.4 ± 0.38	16.7 ± 0.58	
Zinc	10.7–18.4	μmol/L	4	0	7	8	15.0 ± 0.34	15.1 ± 0.52	
Selenium	0.89–2.03	μmol/L	2	0	18	16	1.72 ± 0.04	1.71 ± 0.06	
Magnesium	0.74–1.23	mmol/L	52	12[e]	0	0	0.74 ± .007	0.79 ± .011	0.0001

[a] Sample size is n = 46 to 50 values for HIV − subjects and n = 112 to 116 values for HIV − subjects, except for glutathione (n = 17 HIV − subjects, n = 35 HIV + subjects). Superscripts c–e indicate significant difference between HIV + and HIV − prevalence of out-of-range levels at first visit, by Fisher's exact test.

[b] Significance level of repeated measures ANOVA test for differences in means of HIV − and HIV − subjects.

[c] $0.03 < p < 0.05$.

[d] $p = 0.009$.

[e] $p < 0.0001$.

[f] Normal range not established for glutathione.

jointly in a logistic regression (respective p values: 0.0001, 0.14). Neither weight loss nor diarrhea in the preceding 6 months was related to the low serum magnesium among HIV-infected patients.

Micronutrient concentrations above normal ranges were observed in both seronegative controls and HIV-infected subjects (Table 2). At the first visit, more controls than HIV-infected subjects had high levels of vitamin A (38 vs. 17%, p = 0.043) and high copper levels (9 vs. 0%, p = 0.038). More HIV-infected subjects had high free choline levels than controls (30 vs. 10%, p = 0.036), and more had high niacin levels than controls (13 vs. 0%, p = 0.049). Current use of antiviral medication by HIV-infected subjects did not appear related to the frequency of high concentrations of folic acid.

Use of Vitamin Supplements as a Factor in Serum Concentrations

Vitamin supplementation was defined as reported use of multivitamins or any specified vitamins. The relationship between intake of supplements and plasma concentrations is presented for data from the first visit. Results based on second visit data were similar.

HIV-infected subjects who were taking vitamin supplements had significantly higher mean levels of B_{12}, folic acid, B_6, thiamin, pantothenic acid, and vitamin C. We observed no differences by stage of illness. The only significant difference among controls was that those taking vitamins had higher concentrations of vitamin C (p = 0.01) than those not taking vitamins.

Because supplementation was more common among HIV-1 infected subjects than controls, comparisons of mean micronutrient levels were adjusted for supplemental vitamin intake. The findings were modified only slightly: The difference between vitamin E concentrations of HIV-infected subjects and controls attained significance (p = 0.03), while the difference in carotene levels was borderline (p = 0.06).

We also examined the impact of vitamin supplementation on the frequency of abnormal micronutrient levels. In analyses that adjusted for vitamin supplementation, HIV-infected subjects had significantly more high niacin levels, more low carotene levels, and fewer high levels of vitamin A than controls. After adjustment for vitamin supplementation, the prevalences of low- and high folate concentrations were no different among HIV-infected patients and controls.

Prevalence of Low Blood Antioxidant Levels at First Visit

The number of subjects with below-normal blood concentrations of antioxidants (vitamins A, C, E, and carotenes) at the first visit is shown in Table 3, by infection status and stage-of-illness category (seronegative, asymptomatic, symptomatic, AIDS). Eight patients had two low concentrations, only one of whom was taking vitamins. No subject had more than two low antioxidant concentrations, and none had a low vitamin A concentration. Ten of 19 HIV-infected subjects (53%) who were not taking vitamins had at least one low concentration of vitamins C and E or carotenes. Low antioxidant concentrations were more frequent in later disease stages (22% of asymptomatic patients and 46% of symptomatic and AIDS-diagnosed patients). Within each HIV disease category, those taking vitamin supplements tended to have fewer low antioxidant concentrations than those not taking vitamins. Across all four infection and stage-of-disease strata, supplemental intake was a highly significant factor for having fewer low antioxidant concentrations (stratified exact trend test, $p = 0.0006$). Nevertheless, 29% (13 of 45) of HIV-infected patients had low concentrations of at least one antioxidant despite vitamin supplementation.

Glutathione: Correlation with Antioxidant and Magnesium Levels

Plasma glutathione concentrations were positively correlated with concentrations of vitamin C ($r = 0.33$, $p = 0.013$) but were not correlated with vitamins A and E or carotenes. In a stepwise regression of glutathione on these antioxidants, vitamin C was the only significant correlate ($p = 0.0026$). Glutathione and magnesium concentrations were also positively correlated ($r = 0.29$, $p = 0.03$).

Correlation of CD4s and CD4/CD8 Ratios with Micronutrient Levels

For HIV-1 infected subjects, lymphocyte counts closest in time to micronutrient determinations were correlated with the micronutrient concentrations (median interval between lymphocyte and micronutrient assays = 28 days; 90th percentile <90 days). In univariate analyses, higher niacin levels were associated with lower CD4 counts ($r = 0.43$, p

TABLE 3. *Prevalence at first visit of low blood antioxidant concentrations by infection status and vitamin intake*[a]

		Number (%) of subjects			
		Number of low plasma antioxidant concentrations			
Clinical category	Taking vitamins	None	One	Two	Total
Seronegative	No	16 (84)	3 (16)	0 (0)	19
	Yes	12 (92)	1 (8)	0 (0)	13
Seropositive	No	9 (47)	3 (16)	7 (37)	19
	Yes	32 (71)	12 (27)	1 (2)	45
A (asymptomatic)	No	7 (70)	2 (20)	1 (10)	10
	Yes	14 (82)	3 (18)	0 (0)	17
B (symptomatic)	No	1 (20)	1 (20)	3 (60)	5
	Yes	9 (69)	3 (23)	1 (8)	13
C (AIDS diagnosed)	No	1 (25)	0 (0)	3 (75)	4
	Yes	9 (60)	6 (40)	0 (0)	15
All subjects	No	25 (66)	6 (16)	7 (18)	38
	Yes	44 (76)	13 (22)	1 (2)	58
Total		69 (72)	19 (20)	8 (8)	96

[a] Antioxidants included vitamins A, C, and E and total carotenes. Maximum possible number of low antioxidant concentrations = 4.

= 0.0004), and higher biopterin levels were weakly associated with lower CD4 counts ($r = 0.25$, $p = 0.05$). Niacin levels showed a corresponding association with the numeric ratios of CD4 to CD8 counts ($r = -0.48$, $p = 0.0003$). In addition, CD4/CD8 ratios were positively correlated with carotenes ($r = 0.32$, $p = 0.02$) and riboflavin ($r = 0.29$, $p = 0.04$), and weakly related to vitamin B_6 ($r = 0.24$, $p = 0.07$). Significance levels for all other correlations were >0.15.

Table 4 presents the results of multiple regression to select the best joint predictors of CD4 counts and CD4/CD8 ratios among blood micronutrients and trace metals. Niacin and biopterin remained significant inverse correlates of $CD4^+$ counts, and riboflavin was a significant positive correlate. In the best model for CD8 counts, carotenes were a weak negative correlate and vitamins E and B_{12} were weak positive correlates, although jointly these factors explained only 20% of the variation in CD8 (model not shown). Lower CD4/CD8 ratios thus were associated with higher niacin levels and lower riboflavin levels because of lowered CD4 counts; on the other hand, lower CD4/CD8 ratios were associated with lower carotene levels and higher vitamin E and B_{12} levels because of higher CD8 counts.

DISCUSSION

This comprehensive study presents a cross-sectional profile of micronutrient status in a cohort of HIV-infected outpatients and seronegative controls. The cohort includes men and women, spans the three CDC categories of illness, and includes subjects infected by i.v. drug use and homosexual and heterosexual transmission. Subjects' diet and vitamin use were unrestricted. Although the study was not population-based, the observations may be more generalizable to the free-living HIV-positive population than studies that attempt to control intake or that focus on late-stage cases after onset of wasting.

Of the vitamin-mineral concentrations found to be above or below the laboratory normal range and accompanied by consistent and statistically significant differences in mean concentrations between HIV-positive and HIV-negative subjects, five findings stand out: HIV-positives have lower total carotene, glutathione, total choline, and magnesium concentrations, but higher niacin concentrations.

A substantial percentage of HIV + subjects had low plasma antioxidant levels, in particular, low vitamin C and total carotene concentrations. A striking finding was that self-supplementation appears to spare many HIV + subjects from low antioxidant concentrations at all stages of disease. However, 29% of those taking supplements still presented with one or more low antioxidant levels.

These results confirm other investigations (1,3,8) that showed low concentrations of vitamin C, folate, and carotenes among HIV-infected subjects. No below-normal concentrations of vitamin A were observed; this is not surprising since homeostatic regulation prevents low serum retinol concentrations unless there is frank deficiency (22). There is evidence that vitamin A helps to maintain the integrity of mucosal surfaces and that maternal vitamin A deficiency contributes to vertical transmission of HIV-1 (23). That low vitamin A concentrations were not seen could partially explain the low incidence of heterosexual transmission among these HIV-discordant couples.

The reduced glutathione concentrations found among HIV-infected subjects are similar to the results of other studies and are potentially important because glutathione is a cellular antioxidant (24). The significant association between plasma concentrations of ascorbate and glutathione is consistent with a report demonstrating that vitamin C consumption can increase red blood cell glutathione concentrations (25). However, plasma glutathione concentrations may not correlate with red blood cell of tissue glutathione levels. It is also not clear

TABLE 4. *Multiple regression models of $CD4^+$ counts and CD4/CD8 ratios on micronutrient*[a]

Dependent variable	Predictor	Parameter estimate	p
CD4 +	Intercept	1308	0.0001
	Niacin (μg/ml)	−105	0.0006
	Biopterin (ng/ml)	−220	0.0003
	Riboflavin (ng/ml)	1.12	0.0073
	Biotin (μg/ml)	−0.25	0.058
	Total carnitine (μg/ml)	−26.6	0.051
	Vitamin B_{12} (ng/ml)	−0.24	0.093
CD4/CD8 ratio	Intercept	0.73	0.0001
	Niacin (μg/ml)	−0.102	0.0002
	Carotenes (μg/dl)	0.0018	0.0002
	Vitamin B_{12} (ng/ml)	−0.0007	0.0012
	Vitamin E (mg/dl)	−0.25	0.0017
	Riboflavin (ng/ml)	0.0010	0.017
	Total choline (μg/ml)	0.00090	0.015
	Vitamin A (μg/ml)	−0.0033	0.016

[a] Stepwise selection on 22 micronutrients, terminated by backward elimination when $p \geq 0.10$. For CD4 +, $N = 59$, Model $R^2 = 0.39$; for CD4/CD8 ratio, $N = 46$, Model $R^2 = 0.60$.

that increasing dietary glutathione will increase plasma or cellular glutathione or glutathione peroxidase concentrations. Administration of the glutathione precursor N-acetylcysteine to HIV-infected individuals is being investigated by other researchers.

Niacin levels were higher among HIV-infected subjects, both on average and in the proportion with above-normal levels. Furthermore, higher niacin levels were highly correlated with lower CD4+ counts. The significance of this inverse relationship is not clear. We have not found other reports that document this association or that would help explain this finding.

A 20% prevalence of low serum magnesium concentrations among 224 HIV-1 infected patients was recently reported (26), along with a 46% prevalence of low erythrocyte magnesium concentrations. As in our study, $CD4^+$ lymphocyte counts were not correlated with serum magnesium levels, although they did correlate with red blood cell levels (26). However, another study of 31 AIDS patients observed no evidence of abnormal serum magnesium levels (27). Isolated cases of magnesium deficiency in AIDS patients have been attributed to pentamidine therapy (28). Neither antiviral nor prophylactic medications explain the low magnesium levels observed in our patients nor were their low magnesium levels explained by a history of heavy drinking, wasting syndrome, or recent diarrhea.

Magnesium deficiency may affect both antibody synthesis and T cell function (29). In induced magnesium deficiency in rats and hamsters, cardiac myopathic lesions appear rapidly. These cardiac abnormalities are accompanied by a striking increase in production of the macrophage-induced cytokines interleukin-1, interleukin-6, and tissue necrosis factor alpha (30). The number and severity of the cardiac lesions can be reduced by vitamin E administration, suggesting the possible role of free radicals, perhaps resulting from a cytokine-induced inflammatory response (31). Magnesium deficiency in rats also reduces production of glutathione (32). In the present study, there was a weak but significant linear correlation ($r = 0.29$) between low plasma glutathione and magnesium levels.

The multiple signs and symptoms of magnesium deficiency include lethargy, weakness, fatigue, and decreased mentation (33); these are common complaints of HIV-infected patients. We measured plasma concentrations of magnesium, a predominately intracellular cation. Future studies should examine cellular concentrations in lymphocytes and red blood cells, because lower plasma magnesium may reflect a redistribution of magnesium from the extracellular fluid to the intracellular compartment in HIV-infected cells.

There are two additional points about the magnesium findings. First, although about half of our HIV-positive subjects had low plasma magnesium concentrations, the difference between the means of the HIV-positives and HIV negatives was only 0.05 mmol/L. Whether this difference is biologically significant is unclear. Second, our lower limit of normal for plasma magnesium is 0.74 mmol/L. That lower limit is similar to many laboratories, but in others the lower limit is as low as 0.65 mmol/L. If we had used the lower value, all but five of our HIV-positive individuals would have been considered "normal." Thus, HIV-positive persons may have relative deficits of magnesium rather than frank deficiency.

The normal zinc and copper concentrations in these HIV-infected subjects may reflect absence of acute-phase infections at the time of blood sampling, because infection can increase serum copper and decrease serum zinc (34). Other reports of low zinc and high copper levels among HIV-positive patients have noted that levels were marginally abnormal (35,36) or that abnormal levels were also prevalent among controls (37). The finding of normal selenium concentrations is consistent with another study (38). Thus, our results are not at variance with results of other studies.

Clinical stage of illness had little apparent effect on the micronutrient status of the HIV-infected subjects. However, all of our subjects, even those already diagnosed with AIDS, were in relatively good health and functioning well as outpatients, with no clinical evidence that nutrient metabolism was compromised by wasting or severe diarrhea. The sample sizes were not large enough to detect differences in micronutrient concentrations between stage B (symptomatic) and C (AIDS-diagnosed) subjects that might well be modest in a currently healthy population.

High concentrations of vitamin E, total carotenes, and folates were often observed, possibly due to vitamin supplementation by a majority of the subjects. The inverse correlation of biopterin with CD4 counts (Table 4) could be explained by disease progression, which is marked by elevated levels of neopterin (39), a molecule similar to biopterin. High levels of biopterins are also seen during progression

and immunotherapy of various cancers (40). The lower mean total choline concentrations along with normal free choline could reflect inability of HIV-infected patients to convert free choline to lecithin (phosphatidylcholine), an essential component of cell membranes (41). Reduced total choline concentrations in seropositive subjects could contribute to T cell dysfunction (1).

Conclusions about the benefits of multivitamin supplements are limited by the observational design and lack of controlled intervention. We did not ask subjects for information on diet or for dosages contained in their multivitamin or single vitamin supplements. It was thus not possible to quantify intake of micronutrients or to assess the relative contributions of diet and supplementation.

Seronegative controls were not matched demographically to HIV-seropositive subjects. However, they were similar in age and gender. HIV-discordant couples from the transmission study were necessarily opposite in gender, but they were typically of the same race and comparable age, lived together, and presumably shared a similar diet. Controls enrolled from local staff included some, albeit fewer, nonwhite subjects (20%). Thus, ethnic differences between HIV-positives and controls were unlikely to account for observed differences in micronutrients.

Our results provide additional evidence that micronutrient status may be compromised in HIV-infected subjects in the early stages of disease but may be at least partially corrected among some HIV-1 patients by the use of micronutrient supplements. Controlled interventions are needed to determine whether nutritional supplementation can remedy the possible consequences of abnormal nutritional status. It is possible that prolongation of the interval between infection and symptoms observed over the last decade might relate in part to better nutrition or the use of nutritional supplements. That possibility should encourage additional studies of nutritional status and therapy, of the effects of relatively high doses of supplements (in particular, antioxidants), and of the role of glutathione and its precursors. The role of magnesium deficits in HIV infection and the significance of higher niacin and lower total choline levels also merit further investigation.

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